

STATUS REVIEW: NOTE

Work Area: 01 Biological
Author: R.E. Thornton
Period Covered: January - June, 1982

OBJECTIVE:

To assess, in quantitative terms, the biological response to cigarette smoke and its constituents and to develop acceptable cigarettes with minimum biological activity.

PROGRESS:

There are five areas of study:

1. Direct measurement of biological activity in products and the assessment of changes in product variables such as paper permeability, etc.
2. Smoke Inhalation Studies.
3. Development and use of short-term tests related to cancer.
4. Other short-term tests.
5. Bioassay of nicotine.

The work going on in these areas is as follows:

1. Products of reduced biological activity

(a) Empirical approach

The main activity of the year was quite unexpected twelve months ago. Product variables such as paper permeability, tipping ventilation, filter pressure drop and blend composition had been investigated in two skin-painting experiments (B14 and B15). Unfortunately, subsequent to an analysis of the results, errors in the records kept by the contract research laboratory carrying out the study, were detected. 53 animals in study B14 and 146 animals in B15 were incorrectly recorded. These errors have now been corrected and re-analysis is proceeding as a matter of urgency, and is scheduled for completion in July.

(b) Fundamental studies

Studies into the role of tobacco leaf proteins as precursors of mutagenic compounds of high activity are being planned: assays will be carried out by the Ames Test.

2. Smoke inhalation studies

(a) Investigation of product variables

A major project has been the adaption of experimental protocols to permit the examination of low tar products. For many such products,

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changes in the concentration of smoke components (such as nicotine and carbon monoxide) relative to tar, precluded examination by earlier protocols. This work has been concluded and a series of low delivery cigarettes has been examined. Cigarettes containing expanded tobacco are being studied by a number of protocols, including a one day test developed by Battelle (Geneva) for Gallaher Ltd. The activity of sidestream smoke is also under examination: preliminary results suggests different sites in the respiratory tract are affected in comparison to mainstream smoke.

(b) Development of improved techniques for investigating the response of rodents to inhaled cigarette smoke

This project has been abandoned. We came to the view that an eight week rodent (hamster) study was unlikely to give useful information: a two year study would be required. This was considered to be an ineffective way of utilising our limited resources.

3. Short term test related to cancer

(a) Development of mutagenicity assay

The Ames' Test using *Salmonella typhimurium* has now been established at Southampton. Different smoke condensates have been examined in a collaborative test involving the Hamburg, Montreal and Southampton laboratories: the results from the different group were in excellent agreement.

In preliminary experiments, fresh sidestream condensate has been examined for mutagenic activity and has been found to possess similar activity to that observed for mainstream smoke. However, it is expected that this relationship between sidestream and mainstream smoke may vary depending upon smoking conditions, and this is under investigation.

(b) Development of a chromosome damage assay

To fulfill the minimum test battery to ensure an optimum system for detecting mutagens, an in vitro assay to investigate the ability of compounds under investigation to induce chromosome-damage in mammalian cells is recommended. The in vitro human peripheral blood lymphocyte assay system is now under development and will be able to detect the ability of compounds to induce chromosome mutations.

(c) DNA-Binding

In view of the progress that has been made in project (d) below, work on DNA-binding has been given low priority and this project will be reviewed in the Autumn when a biochemist (Dr. P.M.M. Godden) is scheduled to join the staff.

(d) Biochemistry of chemical carcinogens

Studies involving the University of Surrey.

It has been demonstrated that the enzyme ethoxyresorufin de-ethylase (ERD), a Cyto-P443 linked enzyme and part of the microsomal detoxification system, is frequently involved in the activation of chemical compounds to potentially active carcinogens. The general response

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of this enzyme following in vivo administration of a spectrum of structurally related known carcinogens or non-carcinogens has been studied at Surrey. The preliminary findings indicate that the induction of ERD is highly correlated with known carcinogen exposure.

Collaborative studies between GR&DC and Surrey have been concerned with studying the changes in lung levels of ERD together with changes in its kinetic properties following smoke exposure. Initial studies indicate the following relatively short periods of smoke exposure ERD can be induced considerably (6-10 fold) with changes in its kinetic properties. These studies are continuing to investigate (i) the longer time course of smoke exposure (ii) the effect of exposure to different smoke dilution as well as different cigarette types and (iii) changes in the lung ERD activity following exposure to known carcinogens.

4. Other short-term tests

(a) Test related to emphysema

Efforts have been concentrated on studying the retention of nitrogen dioxide, a compound that has been associated with the development of emphysema, work being carried out under contract for the Verband der Cigarettenindustrie. We have also kept in informal contact with similar work being carried out at Papworth Hospital by Dr. T. Higebottam.

(b) Test related to bronchitis

This test was appraised by Dr. F.J.C. Roe in September 1981. He concluded that this technique had merit as a method for quantifying irritation, but was not necessarily related to bronchitis. Work has, therefore, continued and encouraging results obtained. The time course of the response has been established and high sensitivity (to smoke) established. Currently, the dose response curve is being established.

(c) Development of models for atherosclerosis

Work has progressed on the development of a physico-chemical model for modelling atherosclerosis, based on the notion that atherosclerosis occurs in regions of low shear (contract studies, Imperial College, London).

5. Bioassay of nicotine

Demand for this popular assay continues to be brisk. Currently we are attempting to extend the assay to include measurement of a second nicotine metabolite, Nicotine N-Oxide.

6. ZORBONITE

The filter material nitrosobenzene, ('ZORBONITE'), was examined by a battery of tests chosen in consultation with the Group's consultants. Unfortunately, significant biological activity was found in certain tests, notably skin sensitization, and the use of this material was not recommended.

FUTURE WORK

Sidestream smoke will be studied in greater detail, with attention being given to the development of better methods for collecting sidestream smoke. It is also likely that some further work on expanded tobacco will be required, if only to clarify differences in results reported by ourselves and Gallaher Limited.

New studies being considered include work aimed at examining the effects of smoking on weight gain and metabolic rate in humans and collaborative studies into the effects of smoking in animals and humans on lung permeability, the so-called "leaky lungs".

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